

The University of Chicago

THE RELATION OF LIVER STORES TO
THE OCCURRENCE OF EARLY SIGNS
OF VITAMIN A DEFICIENCY IN
THE WHITE RAT

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS
AND HOUSEHOLD ADMINISTRATION

1941

By

SADIE BRENNER

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THE RELATION OF LIVER STORES TO THE OCCURRENCE OF EARLY SIGNS OF VITA- MIN A DEFICIENCY IN THE WHITE RAT ¹

SADIE BRENNER

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ONE FIGURE

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Symptoms which have been suggested as evidences of sub-clinical vitamin A deficiency are nightblindness, lowered vitamin A in the blood, and dryness and roughness of the skin with eruption of papules. Although it is generally recognized that each of these occurs in advanced stages of vitamin A deficiency, there is no agreement as to their relative value in detecting the earliest signs of deficiency.

Since the liver is the body's chief depot for vitamin A, it has been assumed that a certain amount of this vitamin in the liver is necessary to prevent the appearance of these signs. If this is true, a study of the liver stores in relation to the order of occurrence of these several signs would be one approach to the problem of what constitutes the earliest indication of hypovitaminosis A. Since the liver assay is impossible on human beings, it was decided to resort to animal experimentation to throw light on this aspect of the problem.

EXPERIMENTAL

Two series of investigations were undertaken.

Series 1. A study was made of the appearance of the early signs of deficiency in relation to liver stores in 4-week old

¹ Submitted by Sadie Brenner in partial fulfillment for the degree of Doctor of Philosophy in Home Economics at the University of Chicago.

rats. Nine litters (four to eight rats per litter) from mothers fed Sherman's diet 13 were used. Two rats from each litter (one male and one female) served as positive controls. All the rats were fed the vitamin A-free diet². In addition, the controls received butterfat and cod liver oil which were mixed with the diet, the mixture yielding 38 I. U. of vitamin A per gram of food. Individual records of weekly weights and food consumption were kept. One litter was sacrificed each week. Before anesthetizing the animal, the eyes were bleached for 10 minutes by exposure to bright light to insure a maximum concentration of vitamin A derived from the action of light on visual purple (Wald, '35). The body of the animal was then opened, the blood collected, and the liver and eyes removed. It was usually necessary to pool the blood of several animals in order to supply enough for one determination. The whole eyes of all the experimental animals in a litter were pooled and assayed for vitamin A. The livers were analyzed individually. The same procedure was used for the controls. The extracted blood and tissues were analyzed according to the methods of McCoord and Luce-Clausen ('34) and McCoord ('40), using the Evelyn Photoelectric Colorimeter. The macro unit was used for the livers and the micro unit for the blood and retinas. Analysis for carotene was not necessary because previous studies had shown it to be absent from the body of the rats on the diets used. Sections of skin were sent to the dermatology department for histological examination. Measurements of dark adaptation of the animals were attempted but proved to be unsatisfactory due to the lack of a tropistic response of rats to light.

Results of series 1. As shown in table 1, the livers of rats at weaning contained small but measurable amounts of vitamin A (27 units per 100 gm. of liver)³. These were entirely lost,

² The vitamin A-free diet consisted of 18% alcohol-extracted casein, 4% Osborne and Mendel salt mixture, 1% iodized salt, 67% cornstarch and 10% dried yeast (contributed by the Northwestern Yeast Company, Chicago, Illinois).

³ Evelyn Photoelectric Colorimeter Units. All liver, eye and blood values refer to these units per 100 gm. of liver and eye tissue and per 100 cc. of serum, respectively, unless otherwise stated.

however, at the end of 1 week of depletion. The livers of the controls, on the other hand, increased steadily in vitamin A.

The blood and eye tissues, however, still contained appreciable amounts of vitamin A even after 7 weeks of depletion. At this time the litter examined had a blood value of approxi-

TABLE 1

The effects of depletion on the vitamin A content of the livers, blood and eyes, and on growth.

WEEKS ON DIET	NO. OF RATS		LIVER		BLOOD ¹		EYES ²		WEIGHT	
	Defi- cient	Con- trol	Defi- cient	Con- trol	Defi- cient	Con- trol	Defi- cient	Con- trol	Defi- cient	Con- trol
	gm.	gm.	units/100 gm.		units/100 cc. serum		units/100 gm.		gm.	gm.
0 ³	4	4	27	27	8.5	8.5	49	49	40	40
1	5	2	0	236	..	..	107	175	61	59
2	5	2	0	242	12.4	..	68	78	65	91
3	4	2	0	789	10.1	..	95	56	70	118
4	6	2	0	786	9.6	..	36	88	74	143
5	5	2	0	947	7.2	8.6	32	56	75	157
7 ⁴	6	6	0	1149	4.7	15.5	44	93	78	199

¹ Blanks indicate results which were rejected due to the formation of a cloudy solution when SbCl_3 reagent was added to the evaporated petroleum ether residue.

² Two things must be borne in mind in interpreting the data obtained on the vitamin A content of the eyes: (a) the small amount of biological material available; (b) the dependence of the vitamin A content of the eye upon its light adaptation (Wald, '35). The number of eyes used for a single determination was at least four. In order to have a sufficiently concentrated extract of the eye tissues little solvent was used for grinding and washing; consequently the extraction of the tissues may not have been complete. Although the animals in this experiment were placed in the bleaching box for 10 minutes prior to their killing, this does not insure that all the animals were bleached to the same extent due to their varied activity. With these considerations in mind it is believed that the actual figures presented for the eyes can only be used to indicate trends or relative changes.

³ One litter was used for the analyses of the experimental and control groups at zero weeks on the diet.

⁴ Three litters of rats were sacrificed at this time.

mately one-half (4.7 vs. 8.5 units) and an eye content only slightly below that found in the first litter sacrificed (44 vs. 49 units). The blood and eyes of the litter mate controls at 7 weeks showed considerable increases in vitamin A above that found in the 4-week-old rats.

The weight gains of the experimental animals were slight in comparison with those of the controls. The deficient rats continued to gain up to the fifth week of depletion at which time a growth plateau was reached.

A microscopic study of the skin sections ⁴ revealed no difference in either the histological structure of the stratum corneum or the cutaneous glands of the control and experimental animals. The external appearance of the fur was also not markedly different in the two groups of animals. Even in one litter of rats allowed to die of vitamin A deficiency there were no measurable changes in the histological structures of the skin.

The signs of avitaminosis A which were present in the depleted animals were mild. The rats were small in size as compared to the controls; some of them exhibited a thinning of the fur on the head and slight inflammation and crustiness of the eyelids. Few rats showed difficulty in walking. Only one death occurred within 7 weeks, and that on the thirty-first day of depletion.

This experiment, then, showed that in the absence of liver reserves the rat is able to maintain appreciable amounts of vitamin A in the blood and eye, and to make slight gains in weight for several weeks. Due to the rapid loss of the liver stores, however, the purpose of this experiment, namely, to study the relation of liver stores to the order of the appearance of the early signs of vitamin A deficiency, was unfulfilled. Therefore, a second investigation was undertaken in which 4-week-old rats were made hypervitaminotic with vitamin A concentrate and the effects of subsequent depletion studied.

Series 2. Ten litters of rats 28 to 34 days of age were maintained on their stock diet, and fed approximately 14,000 I. U. of vitamin A ⁵ daily for 35 days. The supplement was admin-

⁴ Dr. S. W. Becker of the Department of Dermatology of Billings Hospital studied the skin sections which were placed in Bouin's fixative, cut from paraffin and stained with hemalum, erythrosine and saffron.

⁵ A shark liver oil concentrate furnished by Vitamins Inc., Chicago, Illinois, and halibut liver oil contributed by Mead Johnson and Company, Evansville, Indiana, were used.

istered orally from a calibrated dropper. Two comparable animals received an equal volume of cottonseed oil for the same length of time.

One litter, containing two males and two females, was sacrificed 24 hours following administration of the last dose in order to find the storage resulting from massive feeding. The other nine litters were placed on the vitamin A-free diet. One of these, also containing two males and two females, was sacrificed after 3 days of depletion to determine the rapidity in the rate of loss of liver stores. The remaining eight litters were then classified into twelve groups. Each group, except the last two, contained two males and two females. The basis of selection was such that the average age, weight, and representation from the different litters in the groups were as alike as possible. One group was sacrificed each week for the first 6 weeks of depletion; the time interval between observations was then lengthened in accordance with the rates of depletion. The entire depletion period lasted for 30 weeks, during which time fifty-three animals were examined. Chemical analyses for vitamin A were made of the liver, blood and eyes⁶ as in the earlier series. In addition histological examinations of the livers and retinas were made by fluorescence microscopy (Popper and Brenner, unpublished).

Results of series 2. As shown in table 2, the average vitamin A content of the livers and blood of the animals sacrificed 24 hours following the cessation of massive feeding was large in comparison with that found in the stock animals which received cottonseed oil. Throughout the first week of depletion the liver content of the animals sacrificed showed no reduction⁷. Thereafter the liver stores decreased rapidly. In the males 63% of the original stores was lost by the end of the second week, and 93% by the eighth week. After that the loss was more gradual, 98% of the original stores being lost only

⁶ These eyes were bleached for 30 minutes.

⁷ Since the storage ability of the individual animals varied somewhat, an average of the liver values for the first three groups was used to express the amount of vitamin A storage resulting from massive feeding. The calculations which follow are based on this figure.

at the end of the eighteenth week. The amount remaining in the liver was representative of that found in adult stock animals. The results for the females were similar except that they had a higher vitamin A liver content than did the corresponding males in all groups but one.

The effects of depletion on the blood were more immediate than on the liver stores. Within 3 days the blood level had

TABLE 2

The effects of depletion on the average vitamin A content of the livers, blood and eyes of hypervitaminotic rats¹

DEPLETION TIME	WEIGHT		LIVER WEIGHT		VITAMIN A CONTENT					
					Livers		Blood ²		EYES	
	Male	Female	Male	Female	Male	Female	Male	Female		
	gm.	gm.	gm.	gm.	units/100 gm.	units/total liver	units/100 cc. serum		units/100 gm.	
0 days ³ cotton-seed gp.		164		6.485		252		16		10.5
vit. A gp.	191	135	8.796	6.753	9,133	12,340	794	833		40.0
3 days	185	139	9.290	6.407	7,688	15,649	764	1003		18.7
7 days	201	163	8.398	6.791	13,078	17,096	1105	1222		18.7
Average	192	146	8.828	6.650	9,966	15,028	888	1026		
2 wks.	194	155	8.491	6.351	3,663	6,103	303	388	13.2	11.5 83
3 wks.	278	168	10.625	6.230	5,088	4,419	534	276	19.8	9.4
4 wks.	246	167	8.941	5.962	1,669	3,750	218	150	14.7	8.8 70
5 wks.	251	196	8.619	6.539	1,836	3,783	162	247	17.0	13.1 69
6 wks.	255	173	8.446	5.908	849	3,248	72	182	13.5	7.7 98
8 wks.	314	190	10.089	5.593	684	1,883	69	106	12.4	8.8 35
10 wks.	299	191	9.697	5.418	294	1,115	29	61	15.4	8.6 27
13 wks.	256	181	6.864	5.213	170	1,661	12	85	10.5	6.5 28
18 wks.	272	179	7.366	5.532	159	433	12	24	8.4	6.6 40
23 wks.	270	202	6.926	6.778	80	211	6	14	8.7	6.5 28
30 wks.	322	191	8.028	5.801	50	311	4	20	8.4	8.7 36

¹ Each figure represents the average for the two animals of the same sex except in the last two groups where there are one male and one female, and one male and two females, respectively.

² In the first three groups the blood samples of the males and females were pooled so that no separate values are available for the sexes for these periods.

³ Determinations were made 24 hours after the last dose.

dropped from its initial value of 40 to 18.7 units. Thereafter it continued to fall, but at a much slower rate, until a plateau was reached at about the thirteenth week. If the first high blood value is discounted on the grounds of incomplete removal of vitamin A by the liver, then the average blood level is seen to have decreased from 18.7 to 8.4 units for the males at the thirtieth week of depletion. The average liver stores for that same period dropped from 9,966 to 50 units. The findings

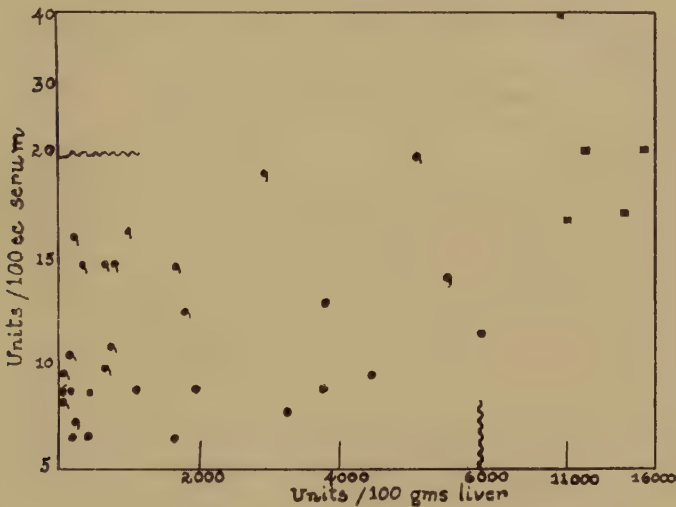


Fig. 1 The relation of blood level to liver stores. Blood values of the males in most cases represent unpooled blood and are plotted against their corresponding liver values; the blood values of the females are usually from the pooled blood of the two females in the group and therefore plotted against their average liver stores. Tagged dots, males; Untagged dots, females; Squares, pooled male and female values.

in the females were again similar but in practically all cases the males had higher blood levels than did the corresponding females.

To determine more clearly the relation between liver stores and blood values, the liver and corresponding blood values for each sex were plotted (fig. 1). A slight tendency toward higher blood values with increasing liver stores during the

early periods of depletion was revealed, especially in the males. However, any one particular blood level covered a wide range in liver stores and, conversely, one liver value was accompanied by a wide range in blood level. For example, a blood level of 8 to 9 units was found with liver stores from 50 to 3,750 units, while a liver storage of approximately 500 units was associated with blood values of 6.5 to 14.9 units.

The vitamin A content of the retina was 83 units at the end of the second week of depletion. At the eighth week there was a decided decrease to 35 units. This approximate level was maintained for the duration of the experiment. A similar amount was found in the eyes of the rats of series 1 after 7 weeks of deficiency.

In this series, the following definite sex differences were observed in respect to storage of vitamin A, rate of depletion and blood levels: (1) On equal intakes, the females had a greater capacity for storage, the males storing two-thirds the amount of the females on the 100 gm. basis. Similar results are obtained on comparing the content of the total livers, though here the difference is not as marked because of the larger livers of the males. (2) The females not only stored more vitamin A, but also retained it more tenaciously (table 2). At the end of the sixth week the males had lost 91% and the females 79% of their original stores; by the end of the thirteenth week the losses were 98% and 91% respectively. At the thirtieth week the livers of the females were found to contain 311 units per 100 gm.; those of the males only 50 units, or approximately twice that in stock rats at weaning. (3) In keeping with their greater capacity for storage and greater ability to retain their stores, the females also had lower blood levels than the males, suggesting a more conservative utilization of vitamin A by the various organs supplied by the blood. To determine the significance of these findings Fisher's t-test was used and the difference found to be significant; these sex differences would have occurred by chance less than one time out of one hundred.

DISCUSSION

Liver stores in 28 day-old rats of diet 13 stock animals disappeared within 1 week whereas the stores of hypervitaminotic rats were still partially filled after 30 weeks on a depletion diet. These rapid losses in young rats have not heretofore been reported. Horton et al. ('41) found almost complete loss in 3 weeks, and Popper and Greenberg ('41) in 2 to 3 weeks depending on the weaning age. Such discrepancies may be explained partly on the basis of the varying nutritional history of the rats and on the different methods used for analyses of liver stores. The rapidity of loss of the excess liver stores of the hypervitaminotic animals confirms the early work of Davies and Moore ('35). The rates of loss in their study were identical with those found in this study through the eighth week of depletion; from then on the rate was slower in the present study. Moore ('40), in later work, failed to confirm the rat's lack of economy in utilizing its excess stores. He attributed the rapid loss obtained previously to a lack of vitamin E in the depletion diet since vitamin E seems to influence the vitamin A storage in the liver (Moore, '40; Bacharach, '40). In the present experiment the animals were kept on diet 13 for the length of the entire supplementary feeding period and it would be expected that enough vitamin E was stored so that the early phase of depletion was not affected.

Although the vitamin A content of the blood and eyes of hypervitaminotic rats decreased from their original values during the process of depletion, the ability of the organism to maintain significant amounts of vitamin A in these tissues in spite of continued absence of liver stores was demonstrated over a period of several weeks. This confirms quantitatively the work on the retina by Greenberg and Popper ('41) who used fluorescence microscopy. In the light of these findings one may question the value of dark adaptation as an indication of one of the early signs of vitamin A deficiency.

The persistence of vitamin A in the blood and eyes, in addition to the small gains in weight made by the animals with no liver stores, suggests the possible presence of vitamin A

in a usable form in other body tissues, thus helping to keep vitamin A in the blood and eye during depletion until these "emergency" stores are used up. This agrees well with Dann's work ('32, '34) which showed that there was no correlation between the vitamin A liver stores present at weaning and the survival time. He suggested a tissue vitamin A not as yet chemically detected. Popper and Greenberg ('41) have demonstrated that the kidneys and adrenals are the only organs other than the retina which retained vitamin A in the absence of liver stores. Horton et al. ('41) did not find vitamin A in the blood when liver stores were empty.

In this study the blood level was found to be of slight value as an index to the nutritional status of the rat except perhaps in extreme deficiency and immediately after massive feeding. These states of vitamin A nutrition were accompanied, respectively, by extremely low and high blood levels. The diagnostic value of intermediate blood levels was by no means clear-cut. All that could be concluded was that higher blood values than those obtained in extreme deficiency, although they tended to be somewhat proportional to liver storage at the upper ranges, can only be interpreted as meaning that stores are present in other parts of the body (i.e., kidney and adrenals) and not as indicating the quantity of reserves in the liver. Closer relationships were obtained between the blood level and liver stores by Horton et al. ('41) and Lewis, Bodansky et al. ('41). A possible cause of these discrepancies is found in Leong's work ('41) which showed that the type of relationship depended upon whether the graded feeding or the depletion technique was used. Positive correlations were obtained with the former, no correlation with the latter.

Sex differences in the vitamin A content of the liver and blood and in the rate of loss of liver stores have previously been reported. From her analysis of the figures of Lagoras and Drummond on control animals, Kimble ('39) has pointed out a sex difference in favor of the females in the ability to store vitamin A. She has also found in human beings that males have a significantly higher blood level than females.

Murrill et al. ('41) have substantiated this conclusion. Bult and Sorgdrager ('38) found a slower rate of loss of vitamin A stores in female and castrated male rats than in normal males. The higher blood level and lower vitamin A stores of the rats may be taken as an indication of a more rapid dispersal of the liver stores, in contrast to the greater economy in utilization by the female. This is not surprising in the light of the role of the female in reproduction and lactation.

It would seem then from this study, that in the rat the use of the vitamin A content of the blood and retina or changes in the histological structure of the skin as early detectors of vitamin A deficiency is not warranted if by deficiency is meant the lack of liver stores. All three of these measurements were resistant to change even in the absence of liver stores. However, it was shown that, although a blood value for a particular rat could not be used to estimate the quantity of its liver stores, the blood value might have some diagnostic significance for large numbers of rats, where in general it could be assumed that higher blood levels were more apt to be associated with higher liver storage than were low blood levels.

SUMMARY

A study of the vitamin A liver stores in relation to the appearance of several alleged subclinical signs was undertaken in rats on a vitamin A-free diet. It was found that:

(1) Whereas 4-week-old rats lost their total vitamin A liver stores after 1 week, hypervitaminotic animals lost 90% of their excess stores after 8 weeks and 98% after 18 weeks. At the thirtieth week of depletion the liver still contained vitamin A.

(2) The vitamin A content of the blood declined rapidly from the hypervitaminotic level, and then decreased more gradually until a plateau was reached for both sexes at about the thirteenth week.

(3) The vitamin A content of the retina decreased to the eighth week of depletion when a plateau was reached.

(4) In the absence of liver stores for several weeks vitamin A was still present in the blood and eye in amounts found in stock rats of weaning age.

(5) A significant sex difference existed. The females stored and retained more vitamin A in the liver than the males, while the males had higher blood levels.

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